47%, CR3 or greater 18%, and not in remission in 11%. The median TNC infused was 4.06 × 10^7/kg (range, 0.73–28 × 10^7/kg), median CD34 cells infused 2.3 × 10^5/kg (range, 0.3–31.7 × 10^5/kg). HLA matching was 6/6 (23), 5/6 (31), and 4/6 (14). Conditioning was TBI-based in 66% and non-TBI in 34%. GVHD prophylaxis was cyclosporine and steroids in 91%, cyclosporine and Methotrexate +/- steroids in 7%, and other in 2%. Median engraftment times were 23.5 days for neutrophils and 65 days for platelets. Acute GVHD had a median day of onset of 25 days (range, 10–93 days), with grade II-IV seen in 49% and grade III-IV in 25% of patients. The pattern of organ involvement with GVHD is shown in the TableTable below. Isolated GI involvement was seen in 23% of patients with grade III-IV acute GVHD. Event free survival at 1 and 2 yr were 62% and 58% for patients without acute GVHD, 75% and 56% for grade II only, and 53% and 47% for grade III-IV acute GVHD. Survival with higher grade GVHD may be related to both the more delayed onset of GVHD and more manageable course of GVHD after UCBT.

Pattern of organs involved by acute GVHD

		Organ system involved (%)		
Acute GVHD maximum grade	No. of patients	Liver	GI	Skin
Grade I	3	0	0	100%
Grade II	16	6%	50%	87%
Grade III	12	16%	100%	66%
Grade IV	5	80%	100%	100%
Total	36			

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PHOTOCHEMICALLY-TREATED (PCT) SENSITIZED CYTOTOXIC T CELLS AND ANTI-NK1.1 ANTIBODY PRE-TREATMENT PROMOTE BOTH T AND B CELL RECONSTITUTION IN NON-LEAKY ARTEMIS DEFICIENT MICE WITH SEVERE COMBINED IMMUNODEFICIENCY DISEASE (SCID)

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Artemis-deficiency results in T-B-NK+ SCID. It has a high incidence in Athabascan-speaking Native Americans (SCIDA), and is associated with increased sensitivity to alkylating agents and ionizing radiation. We previously reported a murine model of Artemis-deficiency (mArt-/-) that unlike children with SCIDA, had a leaky phenotype with some T cell numbers and function. We backcrossed this mutation onto C57BL/6 (B6) mice and compared the phenotype to mArt-/- 129/SvJ "leaky" mice. We found that the %129/SvJ in the background significantly correlated with increasing leakiness. B6 mArt-/- mice have virtually no leakiness based on phenotyping and function, comparable to SCIDA children. When injected into 8 w/o mArt-/- recipients, we found that 1 × 10^5 Balb/c allomismatched lineage depleted and c-kit enriched (lin- c-kit+) hematopoietic stem cells (HSC) were rejected (Table). Anti-NK1.1 antibody pre-treatment resulted in limited engraftment. We wanted to develop a non-chemotherapy based approach to promoting engraftment. We generated Balb/c donor T cells sensitized to B6 mice and treated them with Uvadex and UVA photochemical therapy (PCT) which inhibits proliferation but not cytotoxic function. When 6 × 10^5 PCT sensitized Balb/c CD3+ T cells were co-injected with 1 × 10^5 Balb/c HSC into NK-reduced mArt-/- 8 wk old mice, significant T and B cell reconstitution occurred by 4 wks post transplant with no evidence of GvHD at 8 wks (Table). However, no engraftment appeared without anti-NK1.1 antibody pre-treatment. These results indicate that both PCT sensitized CD3+ T cells and anti-NK1.1 antibody pre-treatment are necessary to enhance the immune reconstitution following an allogeneic HSCT. This non leaky Artemis-deficient mouse model more closely resembles the phenotype seen in children with Artemis deficiency including resistance to mismatched allogeneic cells and is ideal for studying novel ways of correcting the immune deficiency in SCID patients without using conditioning therapy with alkylating agents or ionizing radiation.

Immune reconstitution in Artemis-deficient mice following allogeneic mismatched HSCT

	4 Wks Post HSCT		8 Wks Post HSCT	
Marrow Prep and Pre Treatment (n)	T Cells[1]	B Cells	T Cells	B Cells
HSC (5)[2]	0	0	N/A	N/A
HSC/Anti-NK1.1(5)	0.20 ± 0.1	0.12 ± 0.1	0.51 ± 0.2	0.39 ± 0.2
HSC/PCT sensitized T cells (3)	0	0	N/A	N/A
HSC/PCT sensitized T cells/Anti-NK1.1 (n = 10 at 4 wks and n = 4 at 8 wks)	19.4 ± 11.8	22.4 ± 15.8	57.5 ± 26.7	30.7 ± 26.2

[1]Percent of CD3+ T cells or CD45R/B220+ B cells in CD45+ lymphocyte gate.
[2]HSC = lin- c-kit+ cells; 100,000 cells injected/animal.

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ALLOGENEIC STEM CELL TRANSPLANTATION FOR CHILDREN WITH HEMOGLOBINOPATHY USING REDUCED INTENSITY CONDITIONING

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Background and Objective: Allogeneic hematopoietic stem cell transplantation (HSCT) can establish normal red cell hematopoiesis and control hemoglobinopathy associated organ damage successfully in children. However, HSCT is limited by donor availability, conditioning related toxicities, and graft versus host disease (GVHD). Graft rejection rates are high especially after non-myeloablative conditioning. HSCT is largely restricted to matched sibling donor (MSD) transplants following myeloablative conditioning. A multi-center trial using reduced intensity conditioning (RIC) was developed for non-malignant disorders to decrease

Table 1. *HSCT details and outcome*

Patient	Sex/Age (y)/ Diagnosis	Graft	Nucleated cell dose 10^8/Kg	Follow up (mo)	ANC 500/platelets 50K/%donor	GVHD	Events/Lansky
1	F/12/thalassemia	8/8 BM MSD	2.2	53	17/103/97	No	-/100
2	M/2/thalassemia	8/8 BM MSD	3.4	23	18/25/100	No	-/100
3	M/0.8/thalassemia	8/8 BM MSD	5.8	8	11/22/61	No	-/100
4	M/2.5/Sickle cell D	8/8 BM MSD	3.3	48	13/20/100	No	-/100
5	F/9/Sickle cell D	5/6 UCB URD	0.42	22 (died)	16/-/100	chronic extensive	GVHD, infection
6	M/15/Sickle cell D	8/8 BM MSD	2.31	13	12/65/100	chronic limited	DLI for 53% donor chimerism/100
7	F/17/SCD	6/8 BM URD	3.22	10	11/22/100	No	Pneumatosis, sz (resolved)/90
8	M/11/Sickle cell D	8/8 BM URD	6	9	16/19/97	grade 2 acute	hemolysis, CMV/100
9	M/16/Sickle cell D	6/8 PB URD T depleted	1.1 CD34	6	12/did not drop/0	-	graft rejection/ autologous recovery
10	M/18/Sickle cell D	8/8 BM URD	4	1.5	13/14/97	grade 4 gut (resolved)	GVHD/90